MATING TYPES IN OXYTRICHA AND THE SIGNIFICANCE OF MATING TYPE SYSTEMS IN CILIATES [1]

R. W. SIEGEL [2]

Zoology Department, University of Pennsylvania, Philadelphia 4, Pennsylvania

Sonneborn's (1937) discovery of mating types in *Paramecium aurelia* made possible a new approach to the classical and perplexing problems of sexuality, inheritance, and life cycles in ciliated protozoans. Mating types were later found in other species of this genus (Sonneborn, 1938; Jennings, 1938; Giese, 1939; Gilman, 1939) and in *Euplotes* (Kimball, 1939), *Stylonychia* (Downs, 1952), and *Tetrahymena* (Elliott and Gruchy, 1952; Nanney and Caughey, 1953). The present communication reports mating types in a fifth genus, *Oxytricha*. The mating behavior and some aspects of the life cycle of *O. bifaria* will be described. Finally, speculations as to the functions and significance of the known systems of binary and multiple mating types in ciliates will be presented.

Materials and Methods

Specimens of fresh water collected from natural sources were found to contain the hypotrich, *Oxytricha bifaria*. Four stock cultures, each representing the progeny of a single cell, formed the initial material for this investigation. A list of the stocks and their sources follows: stock 4 from Bloomington, Ind.; stock 14 from Ann Arbor, Mich.; stocks 24A and 24C from Locksley Station, Pa. These cultures can be induced to conjugate with one another and, together with their sexual progeny, furnish nine mating types to be described below. Additional collections provided new stocks belonging to one or another of these mating types.

In general, the methods described for handling and culturing *Paramecium aurelia* (Sonneborn, 1950) were successfully adopted for studies of *Oxytricha*. Mass cultures and isolation lines were routinely maintained in lettuce or Cerophyl infusions inoculated with *Aerobacter cloacae*. Under these conditions the present strains of *Oxytricha* have given, after three years of observation, but few indications of cyclic periods of depression or of senescence and death reported for other pedigrees (see Jennings, 1929). But it is important to note that some stocks and hybrid clones (obtained from crosses between the original stocks) lost their initial vigor, became senile and finally died. To induce conjugation, diverse cultures were provided sufficient nutrient to allow 0.5 to 1.0 fissions per day, and were mixed in a depression slide when they had exhausted most of the available bacteria. Mating began about two hours after the mixture was made; if well-fed cultures were mixed, the occurrence of conjugation was delayed until the food supply had been further exhausted. When one or both of the cultures were allowed longer periods of

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[2] Present address: Department of Biology, Reed College, Portland, Oregon.

starvation before mixture, the cells usually became cannibalistic. Cannibals soon attained abnormally large sizes and could not be induced to conjugate. Under optimal conditions, 25 to 95 per cent of the cells formed pairs. Conjugation can apparently take place at any hour of the day and at temperatures ranging from 17 to 32 degrees C.

Nuclear reorganization in the present strains is apparently limited to conjugation; attempts to induce autogamy by limiting the food supply of mature clones proved unsuccessful.

Results and Discussion

In *Paramecium,* true conjugation can take place only between cells of complementary mating types. But in *Euplotes,* cells of a given mating type will conjugate when exposed to filtrates from cells of other mating types (Kimball, 1939) and, for this reason, mixtures of diverse cultures may come to contain induced "selfing" pairs together with other pairs consisting of cells of complementary mating types.

Table I

The breeding system in O. bifaria

(*The plus signs signify that conjugation normally occurs in mixtures of the types indicated*)

	I	II	III	IV	V	VI	VII	VIII	IX
I	–	+	+	+	+	+	+	+	+
II		–	+	+	+	+	+	+	+
III			–*	+	+	+	+	+	+
IV				–	+	+	+	+	+
V					–	+	+	+	+
VI						–	+	+	+
VII							–	+	+
VIII								–	+
IX									–

* Small numbers of selfing pairs frequently observed.

Pairs formed following the mixture of diverse cultures of *Oxytricha* regularly consist of cells of diverse types. This conclusion is based on the following facts: (1) Animals cultured in lettuce infusions for a few days appear darker than others grown in Cerophyl infusions. Appropriate mixtures of "dark" and "light" cells provided conjugations in which each pair consisted of a dark cell mating with a light cell. (2) Soon after they join, pairs may be forced apart by repeatedly expelling them from a micropipette. The mating types of members of such split pairs were always found to be complementary. (3) Attempts to induce "selfing" by exposing cells to filtrates from cells of other types have failed.

However, not all conjugations in *Oxytricha* involve cells of complementary mating types. Some cultures may self whenever conditions for mating are proper. For example, cultures of stock 24A, mating type III (see Table I) regularly contain about one per cent mating cells. The progeny of split pairs obtained from such type III cultures behave as the parental culture; each new culture will self, but is predominantly type III. Hence, it is possible that selfing pairs consist of cells of the same type induced to mate by circumstances as yet unknown. Alternatively, selfing cultures may consist of a small number of cells of an unstable mating type complementary to the predominant and more stable type, a situation which would result in selfing.

The remarkable mating behavior of *Oxytricha* prior to conjugation merits description despite similarity to the "Paarungsspiel" previously described for *Stylonychia*, a closely related genus, by Grell (1951). When cultures of complementary types are mixed the activity of the cells remains unchanged for about two hours. Thereafter, small groups of cells collect on the bottom of the culture dish and within each group the cells describe contiguous circles. Soon two such "dancing" cells meet at their anterior adoral surfaces and become relatively quiescent. The mates so joined may presently separate and find other partners or become more firmly united. If conjugation proceeds each cell is supported on the bottom of the dish by its posterior cirri, and the pair forms an obtuse angle. This angle is slowly reduced so that the cells finally adhere tightly along most of their lengths and conjugation is completed in this position. Mating behavior in *Euplotes* has been found to conform in general to the description given above (Kimball, personal communication; Siegel, unpublished). The complicated pattern of mating behavior observed in these organisms suggests that such cells bear a "sticky" stuff on localized surface areas which must be brought together in order to initiate conjugation. In contrast, other ciliates such as *Paramecium* form large clumps of mating cells; these animals are apparently sticky all over, initial unions are non-specific, and mating behavior is comparatively simple and direct.

Nine mating types are presently known, although relatively few stocks and hybrids from but two crosses have been studied as yet (Table I). Each type will conjugate with all of the other eight types but, with the exception of type III, will not self in unmixed controls. It seems quite likely that new stocks and other hybrids will provide additional mating types. Whether the species *O. bifaria* encompasses genetically isolated subspecies or "varieties" at least superficially comparable to those in *Paramecium aurelia* and *P. bursaria* remains to be discovered.

Some crosses (I × II, III × V, V × VII) yield high proportions of viable hybrids while the majority of the hybrids of other crosses (I × III, II × V) die. Hybrid mortality could be due either to strain differences or to the incompatibility of certain mating types *per se*. In contrast to these results, selfing is almost invariably followed by death, even in the case of cultures which can be successfully outcrossed. This fact suggests that lethal factors, responsible for mortality in both selfing and certain outcrosses, may be discovered in further genetic analyses of breeding relations.

Oxytricha exemplifies the ciliate life cycle first described in Maupas' (1888; 1889) monumental papers and intensively studied by Jennings (1944) in *Paramecium bursaria*. Starting as exconjugants, clones pass through stages of immaturity, adolescence, maturity, senescence, and finally die. Following the separation of conjugants, the new clones undergo periods of vegetative reproduction during which they cannot be induced to mate and hence are called "immature." Adolescence is marked by the ability of the new clone to conjugate with some, but not all, of the established mating types. Moreover, when these cultures can be induced to mate they yield relatively small numbers of conjugating cells. The stages of immaturity and adolescence may last for only a month or may persist for as long as two years. After further reproduction the clone acquires the capacity to mate readily with representatives of all the mating types, save those of its own type, and is now mature. A few hybrid cultures have passed from maturity to senescence and death, but most remain mature for at least two years. The persistence of each of these life cycle stages reflects a pattern of changing cell heredity or progressive differentiation under the control of genetic factors which are as yet unknown. In view of the various hypotheses for the general phenomenon of aging (Jennings, 1929; Sonneborn, 1954; 1955), it would be of interest to re-investigate this well-known problem, taking advantage of controlled conjugations between strains characterized by dissimilar life cycles.

The life cycles of certain ciliates may be greatly lengthened or perhaps eliminated entirely with the discovery of proper nutritive media and culture conditions. For example, clones of *Tetrahymena* do not normally pass through senescence and die off, although, parenthetically, they do undergo definite stages of sexual immaturity followed by adolescence and maturity. The following discussion pertains to other ciliates which show a more or less well-defined and complete life cycle. The important point for our purpose is that under conditions in which such a complete life cycle appears, fertilization has a rejuvenating effect. In these organisms senescence and death seem inevitable in the absence of nuclear reorganization, either uniparental autogamy (perhaps described as endomixis in some forms) or conjugation. If clones are aged, conjugation results in the death of most or all of the enconjugants (Jennings, 1944) and rejuvenescence occurs in only a few or none of the exautogamous progeny (Pierson, 1938; Sonneborn, 1954). Therefore, the periodic occurrence of some form of nuclear reorganization resulting in the formation of new clones would appear essential for the successful maintenance of such ciliate species. Moreover, since these processes may provide novel genotypes and genetic recombinations, they could be of some evolutionary consequence. The development of mating types must have been influenced by these fundamental factors. The significance of systems of binary mating types, as in *P. aurelia* and *P. caudatum*, and multiple mating types, as in *P. bursaria* and *Oxytricha*, will be considered in the next paragraphs.

All else being equal, species with a system of multiple mating types would be expected to conjugate more frequently than species with a system of binary mating types, for contacts between cells of complementary mating types would occur more often. The inadequacy of conjugation as the sole mechanism for nuclear reorganization in species with systems of binary mating types may have led to the development of a process for uniparental nuclear reorganization, namely, autogamy. Or the reverse may have occurred; species which failed to evolve autogamy developed compensatory systems of multiple mating types. At any rate, the two species of *Paramecium* for which binary mating types are known (*P. aurelia* and *P. caudatum*) pass through autogamy, whereas in those three species with multiple mating types (*P. bursaria, P. multimicronucleatum, P. trichum*) autogamy is extremely rare or lacking. Autogamy has not been reported to date in *Euplotes patella, Stylonychia putrina,* or *Oxytricha bifaria* despite intensive efforts, in some cases at least, to detect its occurrence. These species manifest multiple mating types. The available data thus agree with the interpretation of a causal relationship between the kind of mating system and autogamy. Clearly many more species must be investigated before this hypothesis can be adequately evaluated. For example, it would be important in this connection to discover mating systems for *Stylonychia pustulata* and *Paramecium polycaryum*, species in which autogamy has been described by Fermor (1913) and Diller (1954), respectively. On the other hand, one of the six known varieties of *P. bursaria* is enigmatic, for only two mating types have as yet been collected from natural sources. Furthermore, endomixis has been reported for certain cultures of *Oxytricha bifaria* by Kay (1946); massive conjugation epidemics could be induced in wild cultures and mating was regularly preceded by the appearance of a few scattered animals described as endomictics. Unfortunately, this worker was unable to demonstrate mating types among these and other cultures.

Finally, it should be emphasized that although ciliates such as *Tetrahymena* do not pass through stages of senescence and death under artificial laboratory conditions, they may complete the life cycle in natural habitats. If so, the interfertile clones of *Tetrahymena pyriformis* studied by Nanney and Caughey (1953), which form a pattern of multiple mating types and do not pass through autogamy, would lend support to this hypothesis. The fact that *Tetrahymena rostrata* undergoes autogamy (Corliss, 1952) invites an investigation of the mating reactions in that species.

An entirely different explanation for the alternative systems of mating types should be considered. Multiple mating types may occur among species whose natural populations are small, thereby enhancing the chances for cells to encounter suitable mates. On this hypothesis species with binary mating types would be expected to be relatively more populous. However, adequate population studies have not yet been made and the significance of mating systems remains a matter of speculation.

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Summary

A study of *Oxytricha bifaria* revealed a system of nine interbreeding mating types. The mating behavior and life cycle of this organism are described. Two hypotheses which account for systems of multiple mating types in some ciliates and binary mating types in others are presented.

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